ELECTRONIC SUPPLEMENTARY MATERIAL

The role of feeding morphology and competition in governing the diet breadth of sympatric stomatopod crustaceans

Maya S. deVries

Department of Integrative Biology, University of California at Berkeley, Berkeley, CA 94720 U.S.A.

Corresponding author's current mailing address and information:

Marine Biology Research Division
Scripps Institution of Oceanography
University of California, San Diego
9500 Gilman Drive #0202
La Jolla CA, 92093-0202
Email: mdevries@ucsd.edu

Keywords: trophic ecology, mantis shrimp, stable isotopes

SUPPLEMENTARY METHODS

Study site and sample collection

This study was conducted on the reef flat at the Gump South Pacific Research Station, University of California, Berkeley in Mo'orea, French Polynesia (17° 29' 25.5" S, 149° 49' 35.0" W) between November 23-27, 2009. Both the smasher, *Gonodactylus childi*, and the spearer, *Raoulserenea* n.sp., co-occur in the same coral rubble habitat and live in coralline algae nodules and coral rubble crevices. Seven individuals of each of these two stomatopod species were collected by hand on snorkel for stable isotope analysis. Eight potential prey types were also collected: alpheid shrimp (*Alpheus* spp.), clams (Veneridae spp.), crabs (Xanthidae spp.), hermit crabs (*Calcinus guamensis*), fish (*Dascyllus aruanus, Eviota* spp., *Rhinecanthus aculeatus*), planktonic crustaceans (mostly Mysida spp.), snails (*Drupella margariticola*, *Cerithium* spp. Triphoridae spp.), and worms (Eunicidae spp.) (6-15 individuals per prey type, Table 2; taxa identification was based on the Moorea Biocode Project). Alpheid shrimp, clams, crabs, hermit crabs, snails, and worms were collected from the same coral rubble pieces in which stomatopods were found. Fish and plankton were collected with hand nets, though some fish were also found in the coral rubble. Prey types were chosen based on [1], and it was assumed that both species of stomatopods would not consume prey that were much greater than roughly twice their maximum body sizes (*G. childi*: 27.28 mm, *Raoulserenea* n.sp.: 28.34 mm; a 65.21 mm long worm was the maximum prey size). All collections were approved by the Délégation à la Recherche de la Polynésie Française and fish collections were approved by the University of California, Berkeley Animal Care and Use Committee (Protocol #R222-310).

Sample preparation

Upon collection, all animals were frozen and stored at -20°C until they were dissected and prepared for stable isotope analysis. Before dissection, the animals were thawed, but kept chilled. Except for plankton, total body lengths of all animals were measured to the nearest 0.01 mm with digital calipers (*G. childi*: mean ± SD = 25.63 ± 3.61, range = 20.68-28.34 mm; *Raoulserenea* n.sp.: mean ± SD = 23.37 ± 2.61, range = 21.45-27.28 mm). Prey items ranged in size from a 1.10 mm hermit crab dissected from its shell to a 65.21 mm worm.

Hemolymph and muscle tissue from both stomatopod species were dissected following [2], but hemolymph was not well-preserved. Thus, only muscle tissue was analyzed. Muscle tissue was dissected from abdominal somites 2-6. For all prey items except plankton, muscle was dissected and separated from the gut to prevent contamination from stomach contents. Planktonic organisms were analyzed as whole body samples combined into replicates of 0.5 g wet weight of plankton.

All samples were freeze-dried for 48 hrs and homogenized before analysis. Samples were then placed in tin capsules and weighed (Costech Analytical Technologies, Valencia, CA, USA; mean ± SD: 180 ± 50 μg). Continuous-flow isotope ratio mass spectrometry at the University of California, Berkeley Center for Stable Isotope Biogeochemistry was used to analyze carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios. Specifically, elemental concentrations of C and N and stable isotope ratios were analyzed using a CHNOS Elemental Analyzer (vario ISOTOPE cube, Elementar, Hanau, Germany) coupled with an IsoPrime100 Isotope Ratio Mass Spectrometer (Isoprime, Cheadle, UK). Isotope ratios are expressed with delta-notation in parts per thousand (per mil, ‰) as: $\delta^h X = (R_{sample}/R_{standard}-1) \times 1000$,

where h is the high mass number, X is the element, R is the high mass-to-low mass isotope ratio, and $R_{standard}$ is Vienna Pee Dee belemnite (VPDB) for carbon and air for nitrogen. Peach leaves (Standard Reference Material [SRM] No. 1547, n=26, SD of $\delta^{13}C$ and $\delta^{15}N$ = 0.01‰) and bovine liver (SRM No. 1577, n=6, SD of $\delta^{13}C$ = 0.06‰ and $\delta^{15}N$ = 0.03‰) were used as references and standards and to correct for instrument drift and linearity. Samples were analyzed without extraction of lipids or other compounds [2,3].

Data analysis

Analyses were performed using R v. 3.3.0 software [4]. Stable isotope data were tested for normality using the Shapiro-Wilk test before further analysis. Differences in $\delta^{13}C$ and $\delta^{15}N$ values between *Raoulserenea* n.sp. and *G. childi* tissues were evaluated using Welch's two-sample t-test.

To determine the relative consumption of the different prey types in the diet for each of the two species, MixSIAR v. 3.1.6, a Bayesian framework, was used to estimate the proportion of each source (prey) in a mixture (predator diet) [5,6]. This framework allows for variability in predator diet using random, fixed, and continuous effects [7–9], while accounting for uncertainty in trophic discrimination factors (Δ, the difference between the predator and prey stable isotope ratios), concentration dependence (concentrations of carbon and nitrogen [10]), and variability in predator stable isotope values [11,12]. This model is somewhat sensitive to the choice of prior for the residual error. MixSIAR v. 3.1.6 uses a chi-squared distribution with three degrees of freedom for this prior. A later version of MixSIAR uses an uniform prior (0,20) that results in greater uncertainty in the posterior distributions of the estimated proportions of the diet.

Experimentally determined trophic discrimination factors (DF) from the muscle tissue of the smasher species, *Neogonodactylus bredini*, were used in the mixing models ($\Delta^{15}N$= 0.9 ± 0.3 ‰, $\Delta^{13}C$= 3.0 ± 0.6 ‰; [2]), hereafter denoted as "experimental DF's". The results from this analysis were compared to those generated from models using mean DF's that were calculated from literature DF values in mammals, birds, fishes, reptiles, and invertebrates (mean ± standard error from [13]: $\Delta^{15}N$ = 2.75 ± 0.1 ‰, $\Delta^{13}C$ = 0.75 ± 0.1 ‰), hereafter referred to as "conventional DF's." Given that *N. bredini*'s DF values may be species specific and are so different from the convention of $\Delta^{15}N$ = 3 ‰ and $\Delta^{13}C$ = 0-1 ‰ (reviewed in [13]), using both DF's verified that the results were robust to the choice of DF.

To reduce the number of sources in the model (reviewed in [14]), alpheid shrimp and worms were combined *a priori* because their stable isotope values did not differ statistically and the stomatopods handled them similarly ($\delta^{15}N$: $P = 0.33$, $t_{12.05} = 0.88$, $n = 16$; $\delta^{13}C$: $P = 0.57$, $t_{13.89} = 0.33$, $n = 16$) [1,14]. Snails and crabs were also combined ($\delta^{15}N$: $P = 0.97$, $t_{21.42} = 0.04$, $n = 24$; $\delta^{13}C$: $P = 0.34$, $t_{20.19} = 0.98$, $n = 24$). Concentrations of carbon and nitrogen in each prey source were also included in the models (concentration dependence) [10].

An uninformative Dirichlet prior, which assumes a generalist hypothesis that all prey are consumed in equal proportions [1,6], was used in the Bayesian models. Three Markov chain Monte Carlo (MCMC) chains were used to fit the mixing model, and convergence was assessed using the Gelman–Rubin diagnostic [15].

The six prey sources were then aggregated *a posteriori* into hard-shelled prey (clams, crabs, and snails) and soft-bodied prey (alpheid shrimp/ worms, brittle stars, fish) categories [14]. The categories were determined based on observations of prey handling during a feeding experiment conducted in [1].

To quantify diet specialization at the population level for each species, the specialization index, ε (Eq. 5 in [16]), was calculated from the mixing model estimates of dietary proportions. The specialization index ranges from 0 (ultra-generalist) to 1 (ultra-specialist). Mixing model and specialization index results are presented as medians (95 % credible interval, CI).

SUPPLEMENTARY TABLE

Table S1. For comparison to the main model results, Bayesian mixing model median estimates [95 % CI] of the proportional contributions of all eight of the prey types to the 'smasher' and 'spearer' diets are presented. Including all prey sources separately in the models resulted in high uncertainty in the posterior distributions. This was especially true for plankton in the model run with conventional DF's. Thus, unlike with the other models, plankton was likely overrepresented in these model results. Prey are also aggregated *a posteriori* into two categories: soft-bodied and hard-shelled prey (*italics*).

	experimental DF		conventional DF	
prey type	smasher (%)	spearer (%)	smasher (%)	spearer (%)
soft-bodied	*33.6 [12.6, 70.5]*	*76.0 [47.7, 93.9]*	*40.3 [16.5, 82.1]*	*79.6 [51.0, 95.8]*
alpheid	3.8 [0.3, 18.3]	1.4 [0.1, 8.5]	5.9 [0.4, 24.4]	2.6 [0.2, 14.5]
fish	9.7 [3.8, 23.1]	52.4 [28.2, 77.0]	6.8 [0.8, 21.1]	16.1 [0.3, 41.9]
plankton	16.0 [2.0, 45.2]	14.3 [0.6, 54.9]	17.0 [3.3, 33.9]	42.3 [2.2, 65.5]
worm	5.0 [0.3, 20.9]	1.9 [0.1, 10.8]	13.7 [0.9, 40.4]	7.3 [0.4, 33.3]
hard-shelled	*54.2 [12.6, 79.9]*	*17.6 [2.6, 46.8]*	*59.7 [17.9, 83.5]*	*20.4 [4.2, 49.0]*
clam	44.5 [6.9, 68.8]	13.3 [1.5, 37.4]	12.5 [0.1, 38.5]	7.0 [0.4, 46.9]
crab	2.8 [0.2, 12.7]	11.0 [0.1, 6.1]	4.1 [0.3, 19.4]	2.1 [0.1, 14.3]
hermit crab	4.0 [0.3, 15.3]	1.3 [0.1, 6.2]	18.4 [1.4, 40.6]	4.3 [0.5, 14.9]
snail	3.7 [0.2, 16.1]	1.4 [0.1, 9.1]	6.2 [0.4, 26.9]	3.1 [0.2, 23.2]

SUPPLEMENTARY FIGURES

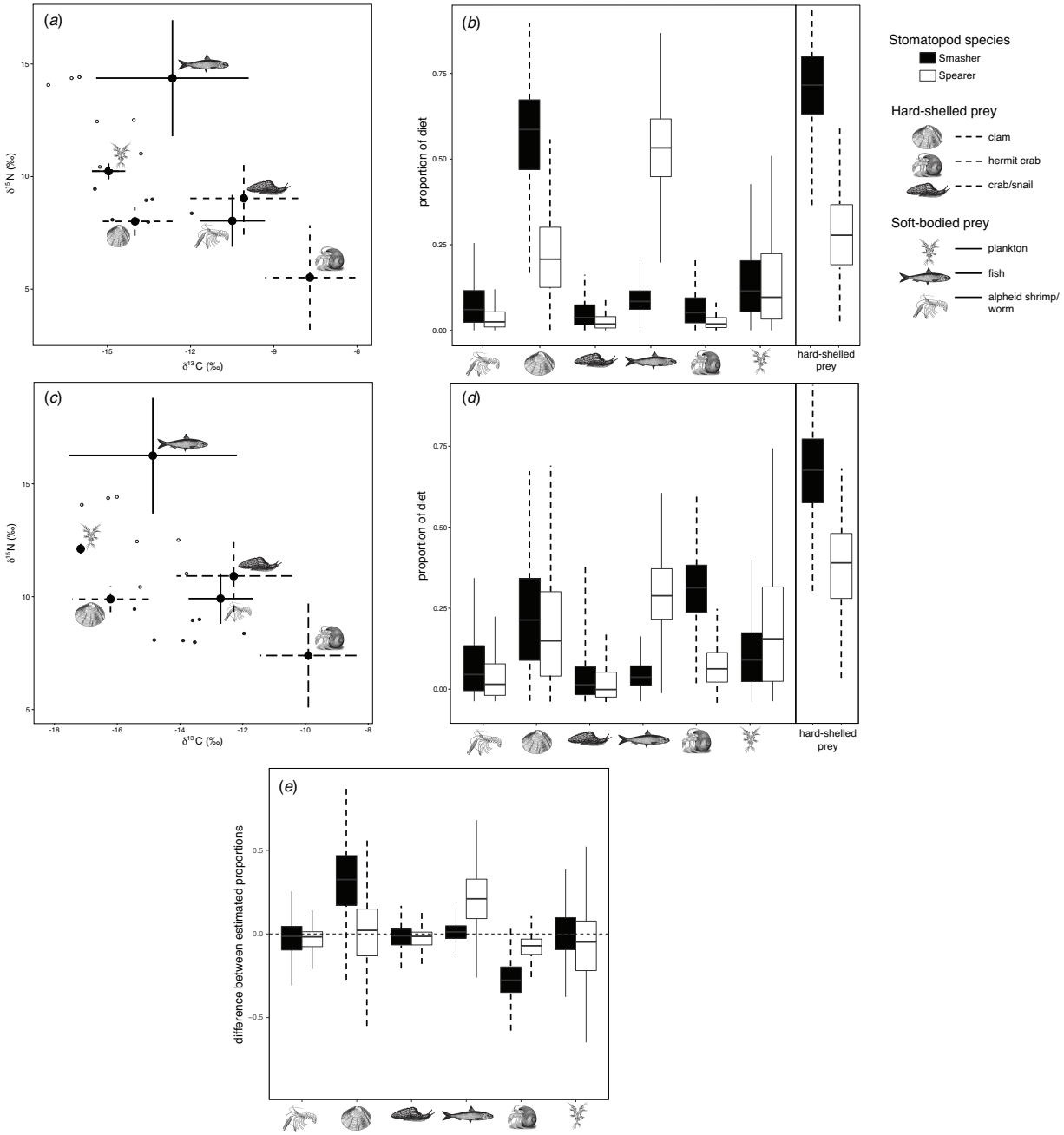

Figure S1. ***(a,c)*** Stable isotope values of the stomatopod species and their prey and ***(b,d)*** mixing model results based on ***(a,b)*** the experimental DF's and ***(c,d)*** the conventional DF's. ***(e)*** Differences between the estimated proportions calculated with the experimental and conventional DF's. Figures ***(a,b,e)*** are repeated here from the main text in order to compare them with the results from the models run with ***(c,d)*** the conventional DF's. For detailed descriptions of symbols and boxplots, see figure 2 in the main text.

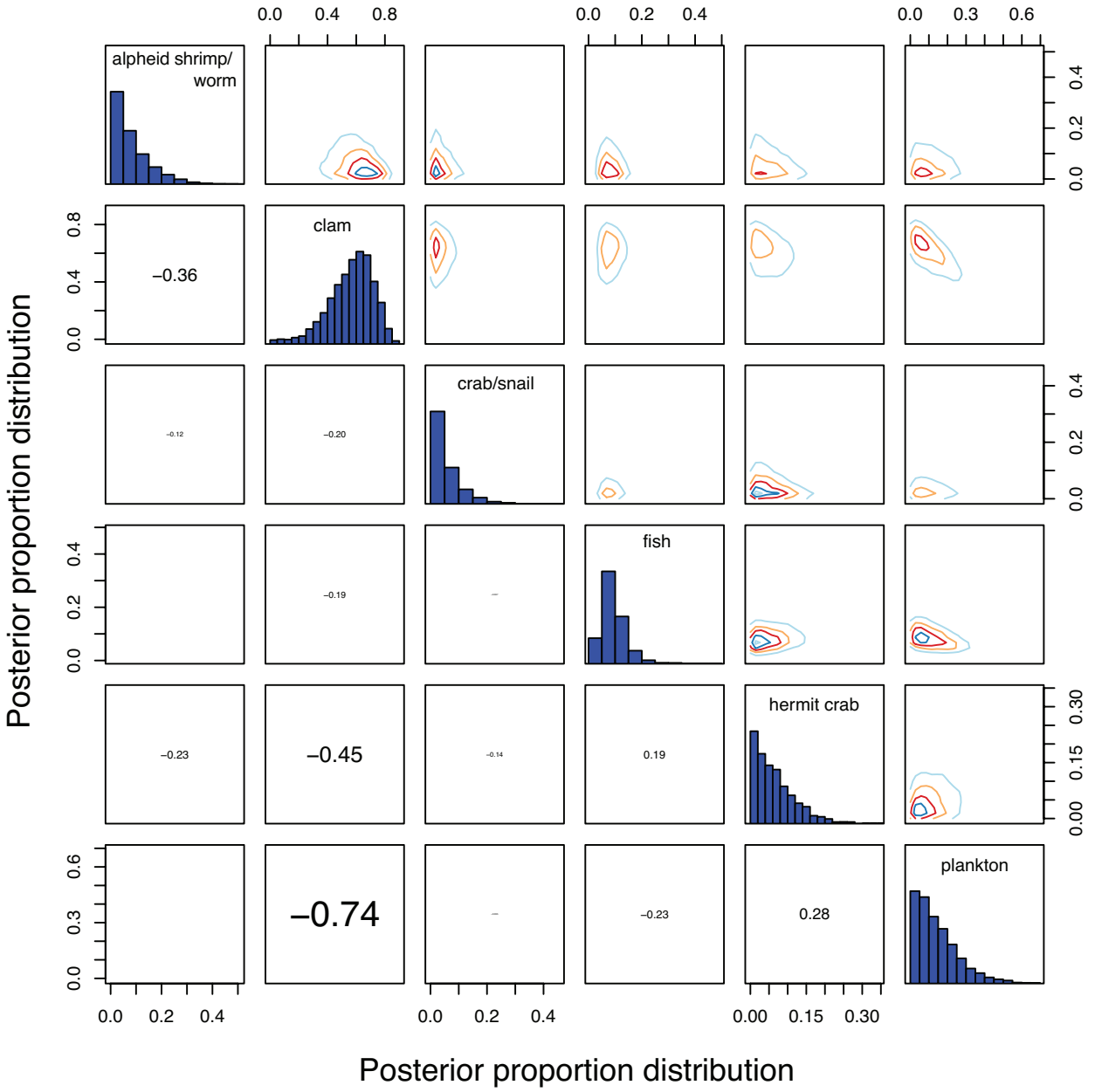

Figure S2. Diagnostic matrix plots or "pairs plots" of the prey diet proportions estimated from the mixing model run with **experimental DF's**. The upper-triangle of graphs has contour plots that show whether pairs of posterior distributions are correlated, the diagonal shows histograms of the proportional contribution estimates, and the lower-triangle shows the numerical correlation coefficients between the different sources. The high, negative correlation coefficients in this model signify that the model struggled to differentiate between clams and plankton. It is therefore possible that clams are over-represented in both the spearer and smasher diets.

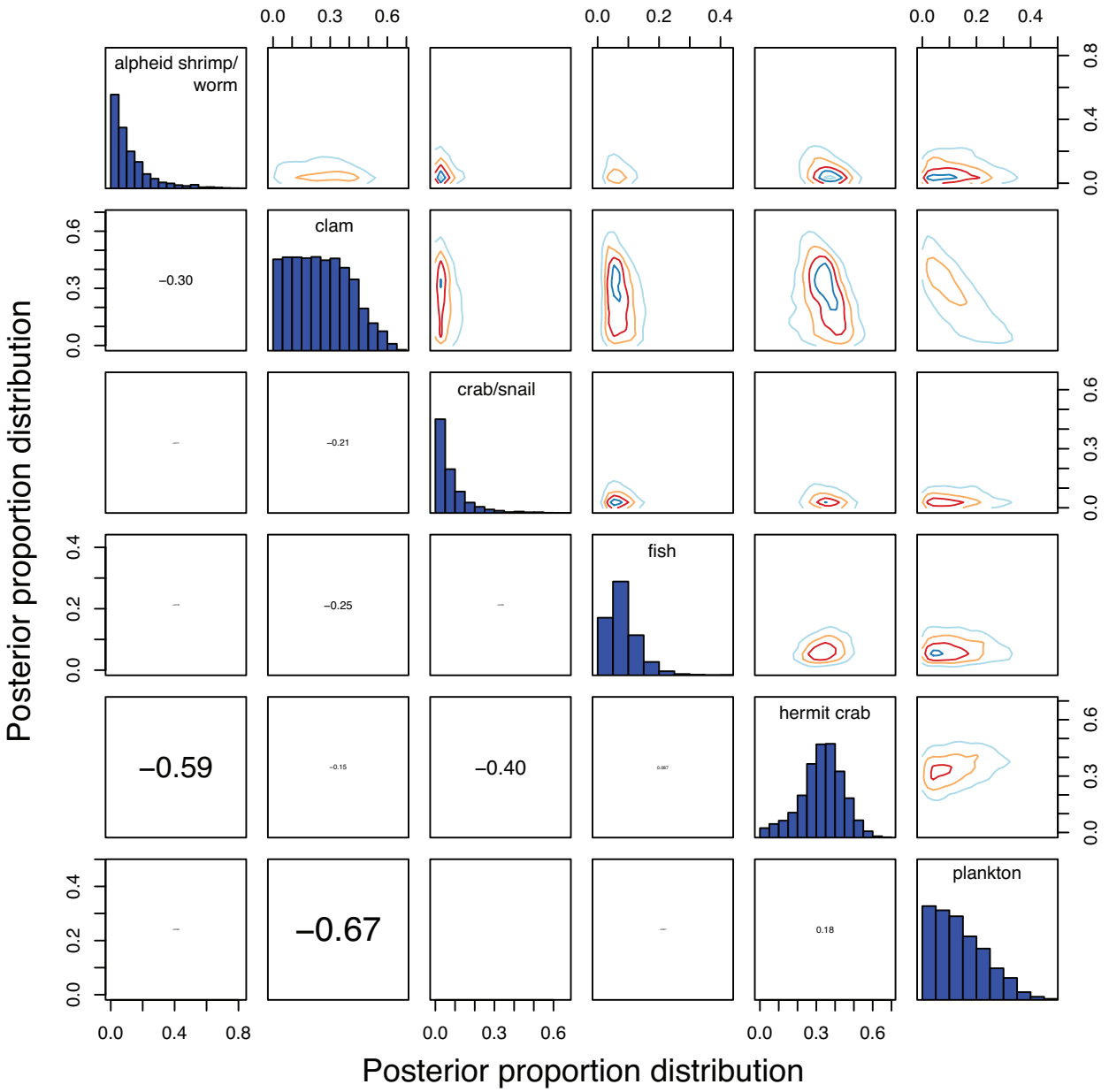

Figure S3. Diagnostic matrix plots or "pairs plots" of the prey diet proportions estimated from the mixing model run with **conventional DF's**. The upper-triangle has contour plots that show whether pairs of posterior distributions are correlated, the diagonal shows histograms of the proportional contribution estimates, and the lower-triangle shows the numerical correlation coefficients between the different sources. The high, negative correlation coefficients in this model signify that the model is struggling to differentiate between clams and plankton and between alpheid shrimp/worms and hermit crabs. It is therefore possible that plankton and alpheid shrimp/worms are underrepresented in the diet because these prey are found in smaller proportions compared to clams and hermit crabs, respectively.

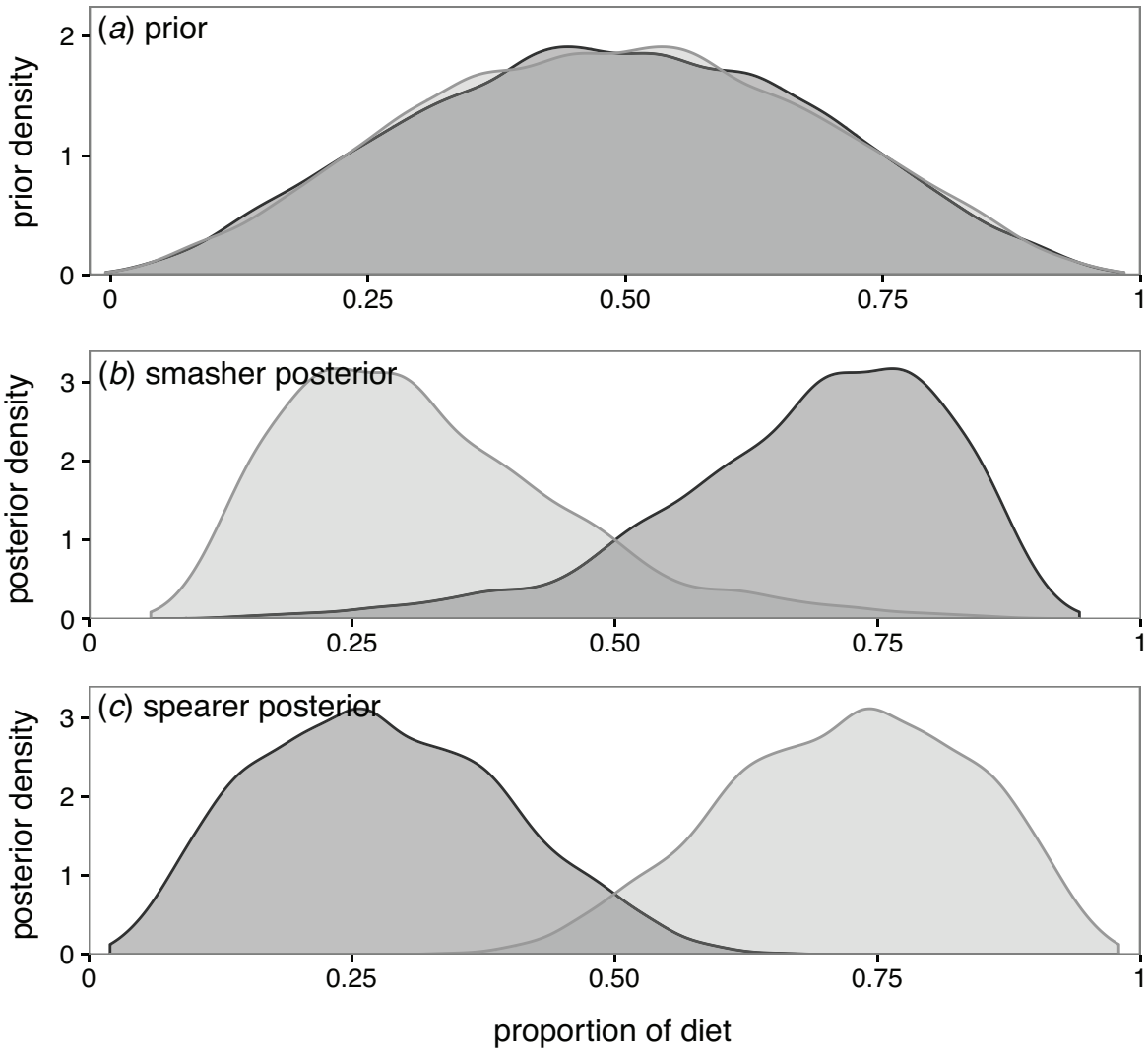

Figure S4. Prior and posterior density plots from the best fit Bayesian mixing model based on runs with **experimental DF's** show the proportional contributions of hard-shelled prey (*dark gray*) and soft-bodied prey (*light gray*) in the smasher and spearer diets. Prey were aggregated *a posteriori* into into categories of hard-shelled prey (clams, crabs/snails, and hermit crabs; $n = 41$), and soft-bodied prey (alpheid shrimp/worms, plankton, and fish; $n = 25$). Despite **(*a*)** the uninformative prior of every prey being consumed in equal proportions, posterior densities from both the **(*b*)** smasher and **(*c*)** spearer show that the smasher consumes mostly hard-shelled prey and the spearer consumes mostly soft-bodied prey. The posterior distributions of the spearer are slightly wider than those of the smasher, which helps to explains why the spearer is not anymore specialized than the smasher.

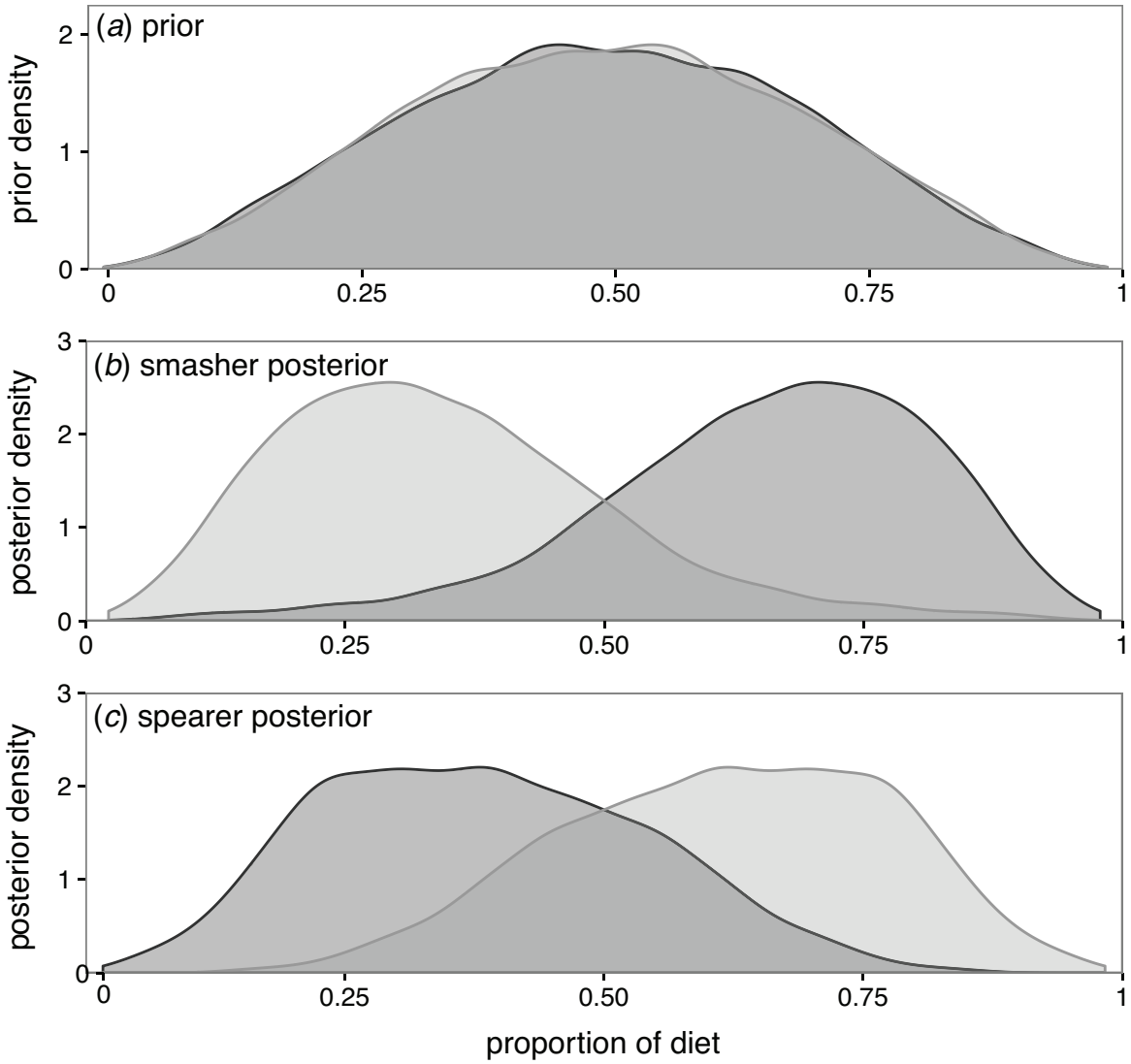

Figure S5. Prior and posterior density plots from the best fit Bayesian mixing model based on runs with **conventional DF's** show the proportional contributions of hard-shelled prey (*dark gray*) and soft-bodied prey (*light gray*) in the smasher and spearer diets. Prey were aggregated *a posteriori* into categories of hard-shelled prey (clams, crabs/snails, and hermit crabs; $n = 41$), and soft-bodied prey (alpheid shrimp/worms, plankton, and fish; $n = 25$). **(*a*)** An uninformative prior was used in the model. Hard-shelled prey was notably present in higher proportions in **(*b*)** the posterior estimates of the smasher diet. Soft-bodied prey was in higher proportions in **(*c*)** the posterior estimates of the spearer diet. The posterior distributions of the spearer are considerably wider than those of the smasher, suggesting that the spearer diet may be wider than the smasher diet in this analysis.

REFERENCES

1. deVries MS, Stock BC Christy JH, Goldsmith GR, Dawson TE. 2016 Specialized morphology corresponds to a generalist diet: linking form and function in mantis shrimp crustaceans. *Oecologia* **182**, 429–442. (doi:10.1007/s00442-016-3667-5)

2. deVries MS, Martínez del Rio C, Tunstall TS, Dawson TE. 2015 Isotopic incorporation rates and discrimination factors in mantis shrimp crustaceans. *PLoS One* **10**, e0122334. (doi:10.1371/journal.pone.0122334)

3. Mateo MA, Serrano O, Serrano L, Michener RH. 2008 Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. *Oecologia* **157**, 105–15. (doi:10.1007/s00442-008-1052-8)

4. R Development Core Team 2014 R: A Language and Environment for Statistical Computing. *R Found. Stat. Comput.* **1**, 409. (doi:10.1007/978-3-540-74686-7)

5. Ward EJ, Semmens BX, Schindler DE. 2010 Including source uncertainty and prior information in the analysis of stable isotope mixing models. *Environ. Sci. Technol.* **44**, 4645–4650. (doi:10.1021/es100053v)

6. Stock BC, Semmens BX. 2013 MixSIAR GUI User Manual, version 3.1, Available at https://github.com/brianstock/MixSIAR. doi:10.5281/zenodo.56159

7. Semmens BX, Ward EJ, Moore J, Darimont C. 2009 Quantifying inter- and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. *PLoS One* **4**, 6187.

8. Francis TB, Schindler DE, Holtgrieve GW, Larson ER. Scheuerell MD, Semmens BX, Ward EJ. 2011 Habitat structure determines resource use by zooplankton in temperate lakes. *Ecol. Lett.* **14**, 364–372. (doi:10.1111/j.1461-0248.2011.01597.x)

9. Parnell AC, et al. 2013 Bayesian stable isotope mixing models. *Environmetrics* **24**, 387–399. (doi:10.1002/env.2221)

10. Phillips DL, Koch, PL. 2002 Incorporating concentration dependence in stable isotope mixing models. *Oecologia* **130**, 114–125.

11. Parnell A, Inger R, Bearhop S, Jackson A. 2010 Source partitioning using stable isotopes: coping with too much variation. *PLoS Biol.* **5**, e9672.

12. Moore JW, Semmens BX. 2008 Incorporating uncertainty and prior information into stable isotope mixing models. *Ecol. Lett.* **11**, 470–80. (doi:10.1111/j.1461-0248.2008.01163.x)

13. Caut S, Angulo E, Courchamp F. 2009 Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol.* **46**, 443–453.

14. Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens, BX, Ward EJ. 2014 Best practices for use of stable isotope mixing models in food-web studies. *Can. J. Zool.* **835**, 823–835.

15. Gelman A, Carlin JB, Stern HS, Rubin DB. 2003 *Bayesian Data Analysis*. 2nd edn. Boca Raton, FL: Chapman and Hall.

16. Newsome SD, Yeakel JD, Wheatley PV, Tinker MT. 2012 Tools for quantifying isotopic niche space and dietary variation at the individual and population level. *J. Mammal.* **93**, 329–341. (doi:10.1644/11-MAMM-S-187.1)